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DESIGNER PIG FEED AND ETHANOL PRODUCTION

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Ethanol Process Test Program

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Designer Pig Feed and Ethanol Production

Final Report

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Executive Summary

Prairie grains contain all of the nutrients required for various types of animals, though rarely in the proper proportions. The basic premise of this project is to create designer animal feeds by fractionating the raw grain into the nutritional components, and then recombining these fractions in the best possible ratios for the specific animal to be fed.

The main components of the process to create these designer-feed streams are a pre-milling plant to separate the raw grain into a fibrous fraction and a starch-rich fraction, and an ethanol plant to utilize the starch that is normally in excess in some of the remaining raw material. The co-products of the ethanol plant contain concentrated essential proteins required for animal feed in the form of fermenter solids and thin stillage.

This test program was designed to test the effectiveness of various premilling methods and validate the operability of a new low-energy ethanol production process for a variety of typical prairie grains of various grades, and to provide nutritional data on the various fractions obtained from these grains. This report contains discussion of the results of the nutrition testing.

The following prairie feed grains were used for this test program: Durum Wheat, Red Spring Wheat, Canada Prairie Spring Wheat, Malt Barley, Shriveled feed grade Barley, Triticale, Yellow Peas, Green Peas, and two varieties of oats. For each of these, chemical analyses for a range of nutritional components including protein, fibre, starch, and essential amino acids were performed on the raw grain, hulls and fibres, starch flour, fermenter solids, and thin stillage. Following are some of the conclusions that were drawn:

Premilling is an effective way to provide significant separation of the fibre and starch in all of the feed grains tested. The proteins preferentially ended up in the milled grain for peas and oats, but did not separate significantly for the wheat, barley, and triticale.

In all cases, there was a significant increase in the concentration of proteins in the fermenter solids and thin stillage compared to the milled grain, although this was not always translated into higher amino acids.

The fermentation and processing methods used in this project have created markedly different co-products from ethanol production than those from traditional processes, creating new opportunities for designer feeds.

There is nutritional value in different forms in all of the prairie grains tested. The added value of these co-products for each grain needs to be weighed against the cost of its processing equipment when determining whether it should be included as a potential feed source.

Multiple premilling steps using different technologies would be beneficial for this project.

Introduction

Background

Although prairie grains contain all of the nutrients required for various types of animals, they are rarely in the proper proportions for any of the animals they are used to feed. Current feed operations are not optimum, in that they invariably overfeed the components that are in abundance (fibre and starch) and require supplements to make up the animal's requirements for those that are lacking (essential proteins). The basic premise of this project is to create designer animal feeds by fractionating the raw grain into the nutritional components, and then recombining these fractions in the best possible ratios for the specific animal to be fed.

The main components of the process to create these designer feed streams are a pre-milling plant to separate the raw grain into a fibrous fraction and a starch-rich fraction, and an ethanol plant to utilize the starch that is normally in excess in some of the remaining raw material. The co-products of the ethanol plant contain concentrated essential proteins required for animal feed in the form of fermenter solids and thin stillage. The fuel-grade ethanol is another valuable co-products of this portion of the process. Following is a summary of the various co-products:

Table 1: Co-Product Summary

Co-product	Source	Primary Component	Optimum Use
Fibres and Hulls	Pre-milling	Fibre	Ruminant Feed
Flour	Premilling	Starch	Monogastric or ethanol plant feed
Fermenter solids	Ethanol plant	Protein	Monogastric feed supplements
Thin stillage	Ethanol plant	Protein-rich water	Monogastric or ruminant feed water
Ethanol	Ethanol plant	Ethanol	Green fuel

Utilization of a new low-energy ethanol production process both reduces operating costs and avoids the nutritional degradation of the fermenter solids and thin stillage that can arise from exposure to the high temperatures that are typically present in ethanol production.

Following are the three main purposes of this test program:

1. Deploy various premilling methods for a variety of typical prairie grains.
2. Validate the operability of the novel ethanol production process using the flour from a variety of typical prairie grains of various grades.
3. Provide nutritional data on the various fractions obtained from these grains.

This report contains discussion of the results of the nutrition testing of the various component streams.

Methods

Feed Grains

The objective of this test program was to determine the nutritional value of various fractions from two different types or grades of five different grains, through premilling and cold hydrolysis ethanol fermentation. There was one exception - due to the typical low availability of triticale and the relatively high importance of wheat, it was decided to experiment with three commonly used types of wheat and only one type of triticale. Table 2 contains a summary of the feed grains used in this test program.

Table 2: Feed Grain Summary

Grain	Bushel weight	Comments
Durum Wheat	58	
Red Spring Wheat	59	#2 grade
Canada Prairie Spring Wheat	59	
Malt Barley	51	
Harrington Barley	41	
Triticale	48	Food grade
Yellow Peas	61	Obtained pre-split, accompanied by corresponding hulls and raw peas
Green Peas	60	
Oats 1	39	Includes wild oats
Oats 2	40	Clean

Premilling Process

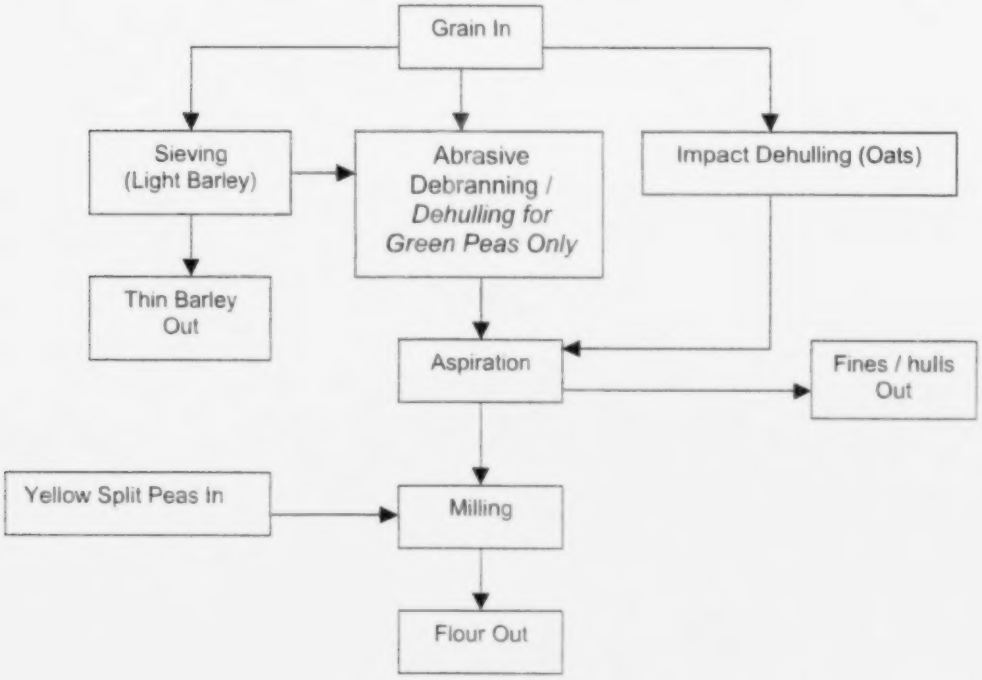
Description

Grain processing was done using equipment in the Department of Agriculture and Bioresource Engineering laboratories at the University of Saskatchewan. It is important to note that these are not all suitable for larger quantities of grain by virtue of their size, and that there are no industrial scale counterparts for some of the devices. This is acceptable since the primary goal of the procedures and milling described herein was to debran grains and mill specific quantities of flour for ethanol fermentation feedstock, as opposed to researching specific methods and parameters

of debranning. For the purposes of designing a full-scale industrial preliminary milling plant, it would be appropriate to conduct any milling method tests on full-size or representatively scaled versions of the actual large-scale equipment being considered in order to obtain meaningful and useful information towards plant design criteria. Grain parameters and handling would also best be more carefully controlled and mechanized (rotary feeder mechanisms, etc) for more rigorous experimentation.

A block diagram of all grain processing is shown in Figure 1. The grains under consideration were put through slightly different process steps according to various necessities. For example, the yellow split peas did not require dehulling. The other notable exception was oats, which were not debranned on account of their high moisture and soft texture, the relatively limited quantity obtained (low density), and their thin, long kernel shape. The light barley was passed over a hand sieve to remove thin kernels prior to dehulling and milling. The rest of the grains were debranned in the abrasive dehuller, aspirated, and milled in batches.

Figure 1: Process Flow Diagram for Grain Processing



Equipment

Abrasive dehulling / debranning was done using a shop-fabricated machine equipped with a manual disc speed adjustment crank. The grains were processed for varying amounts of time as

required to achieve satisfactory fibre removal. Oats were processed in a California Pellet Mill Impact dehuller.

The sieve used to remove thin kernels from the light barley was a 5/64" hand sieve.

Aspiration was done using a Carter-Day laboratory aspirator. This removed fine material and hulls when necessary from the grain stream after the debranning / dehulling step. Air and metering roll speeds were subjectively adjusted for different grains depending on the observed degree of effectiveness of fine material or hull removal.

Milling was done using one of three different machines chosen to meet the feed characteristic requirements of the ethanol plant. The first machine used was a Buhler Hammer Mill, which was easy to use and had a high capacity. When it was determined that the resulting particle size was too large, a Knife Mill with a precision grinder was utilized instead. This mill produced an acceptable grind size of flour, but was very slow and labor-intensive to operate. The final runs were completed using a smaller half-screen hammer mill that was capable of producing a small enough particle size, but had previously been unavailable. It had reasonable capacity, but was more difficult to use than the Buhler Hammer Mill.

Observations

Debranning and aspiration removed 5-10% of the overall mass of the grain bulk, varying slightly between the different types of grain. Another significant percentage of the grain was lost to dust generation, material adhesion to milling equipment surfaces, and small spills - generally between 5 and 10% of the starting grain mass. These quantities were difficult to ascertain with any degree of exactness due to the multiple sources of loss, dust generation, and spillage.

Wheat varieties used had relatively tough bran, and required up to 3 minutes of debranning, whereas the other grains generally showed more bran removal after 2-minute periods. The dehulling of peas proceeded even more quickly, requiring only 45 seconds.

Milling of the oats presented some challenges not encountered with the other grains, due to the higher oil content. The incidence of clogging and sticking increased, causing more frequent equipment shutdowns and making the cleaning between runs more difficult.

Ethanol / Coproduct Production Process

Introduction

Ethanol production was done using an innovative low-energy process developed by Dr. Gordon Hill at the University of Saskatchewan. It utilizes cold hydrolysis in place of the standard fermentation process and liquid-liquid extraction in place of distillation to separate the ethanol

from the water. A further innovation involves using continuous operation instead of the traditional batch operation. This is successfully being done in some industrial applications, and is a recommended practice.

Fermentation

In order to emulate the intended multiple-vessel cold hydrolysis process as closely as possible, the test program utilized two vessels in series, each equipped with three impellers, for the fermentation. Each grain feed was first mixed in a feed tank with the required ingredients, and then fed to the first fermentation vessel at a rate designed to provide about 30 hours of fermentation. A series of level increases and decreases in the two vessels was used at the beginning of each new feed to reduce the mixing between the feeds, to allow distinction between the coproduct analyses.

A constant feed rate was attempted in the series of vessels, so that the feed entering the primary vessel, the feed to the secondary vessel, and the harvest were carried out at approximately the same feed rate. For some substrates, blockages in the feed lines meant that transfer rates had to be manually manipulated, resulting in feed rates that varied from hour to hour, but a reasonable attempt was made to complete the fermentation of each feed in the prescribed amount of time. For all of the pumps, it was not possible to continuously deliver the prescribed feed rate, due to the size of the tubing required to pump the slurry and the minimum rate of the pumps. Therefore, household timers were use to start and stop the pumps so that the appropriate flow rate was delivered, on average, over a one-hour period.

The fermentation conditions for this process are listed in Table 3.

Table 3: Fermentation Conditions

Parameter	Setpoint in 30 L fermenter
Volume	7 to 24 L
pH	4.2
Temperature	30°C
Dissolved oxygen	20% (was uncontrollable throughout fermentation)
Stirrer speed	100 to 130 rpm
Backpressure	0 to 0.5 bar
Airflow	2 to 4.2 sLpm

The fermented material leaving the second fermentation vessel was first collected and then separated by centrifugation into fermenter solids, which were frozen for later analysis, and an aqueous solution of about 5-10% ethanol (beer), which was collected for further processing by liquid-liquid extraction.

Extraction

The separation of the ethanol from the water was done using oleic acid, which selectively attracts the ethanol over the water. After mixing the two phases were allowed to separate, and the water or thin stillage was removed and frozen for later analysis. The oleic acid containing the ethanol was then forwarded to a flash tank, where the ethanol was removed using heat and vacuum. Note that although it was intended to test a process as close to continuous as possible, calculations indicated that this may not provide enough ethanol recovery. It was therefore decided to process half of the substrates using a continuous process, and then half using a multi-stage batch process.

Nutrition Analysis

A summary of the methods used to analyze for the nutritional components of the fractions is given in Table 4. Note that no fibre analyses were done on the thin stillage samples because of the low solids content. Also, starch analyses are not relevant for the fermenter solids or thin stillage, because the starch should be mostly consumed by the fermentation, and interference from other components would outweigh the analysis results at such low concentrations. Amino acid profile tests were not done on any of the raw grains, and only on select bran and fibre streams.

Table 4: Nutrition Analysis Methods

Test	Methodology / Extraction	Instrumentation	Reference
Moisture	Drying 2 hours at 135°C	Oven	Moisture in Animal Feed. (930.15) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
Crude Protein (Kjeldahl)	Digestion in sulfuric acid. Distillation of ammonia and subsequent titration with standard HCl.	Block digester, distiller and automatic titrator.	Protein(Crude) in Animal Feed. Copper Catalyst Kjeldahl Method. (984.13) Official Methods of Analysis. 1995. Association of Official Analytical Chemists. 16th Edition.
Ash	Ash for 2 hours at 600°C	Muffle furnace	Ash of Animal Feed. (942.05) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
Crude Fat	Boiling and extraction of sample in petroleum ether	Soxtec® extraction unit	Fat(Crude) or ether Extract in Animal Feed (920.39) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
Crude Fibre	Digestion of sample in sulfuric acid followed by sodium hydroxide	Labconco® digestion unit	Fiber(Crude in Animal Feed. (962.09) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
Acid Detergent Fibre (ADF)	Digestion of sample in acid detergent solution.	Ankom® fiber analyzer	Fiber (Acid Detergent) and Lignin in Animal Feed. (973.18) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
Neutral Detergent Fibre (NDF)	Digestion of sample in neutral detergent solution	Labconco® digestion unit	National Forage Testing Association. 1993. Neutral detergent Fiber-Amylase Procedure p. 95-103. *In*: Forage Analysis Procedures. NFTA. Omaha, N.E.
Lignin	Digestion of sample in acid detergent solution, followed by extraction with sulfuric acid	Ankom® fiber analyzer, Muffle furnace	Fiber (Acid Detergent) and Lignin in Animal Feed. (973.18) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.
Starch	Megazyme Total Starch Assay Procedure (Amyloglucosidase / α-Amylase Method)	3000-rpm bench centrifuge, 510 nm-spectrophotometer, vortex mixer, standard lab equipment	AOAC Method 996.11, AACC Method 76.13; ICC Standard Method No. 168 (See Literature Cited section)
Amino Acid Profile (Cystine, Lysine, Methionine, Threonine, Tryptophan)	Derivitize with 9-Fluorenylmethylchloroformate and analyze by chromatography	High Performance Liquid Chromatograph with Ultraviolet Detection (HPLC-UV)	Einharsson, S., et. Al. "High-Resolution Reverse-Phase Liquid Chromatography System for the Analysis of Complex Solutions of Primary and Secondary Amino Acids" American Chemical Society, 1986, Vol. 58, No. 8, pp 1638-1643; US EPA 645; US EPA 8318 (See Literature Cited section)

Results

Nutrition Analysis Results

The results from the nutrition analyses are listed in Table 5 on the following page, and shown graphically in Figure 2 through Figure 9 in this section and in Figure 10 through Figure 29 in the Appendix. Following is a Legend for the Sample ID in the table:

Characters / Type of grain:

WD	→ Durum Wheat	WB	→ Red Spring Wheat
BM	→ Malt Barley	BH	→ Harrington Barley
PY	→ Yellow Peas	PG	→ Green Peas
01V	→ Oats variety 1	02V	→ Oats variety 2
T	→ Triticale	WCPS	→ Canada Prairie Spring Wheat

Last two digits / Grain Fraction:

01 → Raw grain
02 → Bran and fibres / hulls
03 → Milled grain (flour)
04 → Fermenter solids
05 → Thin stillage

Figure 2: Raw Grain Fibre and Protein

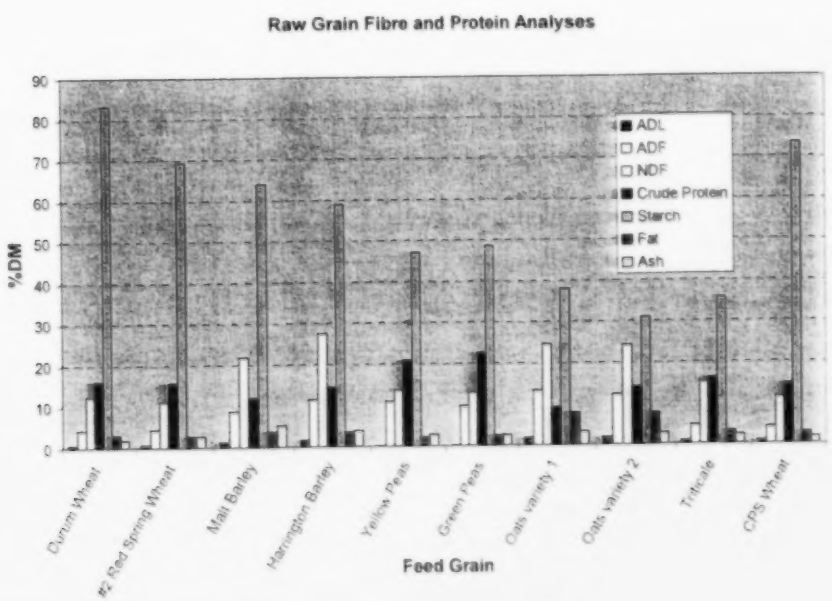


Table 5: Nutrition Analysis Results

Sample ID	Analyses												
	Moisture (both labs)	ADL	ADF	NDF	Crude Protein	Starch (SRC)	Fat	Ash	Amino Acid Profile				
									Cystine	Lysine	Methi-onine	Threo-nine	Trypto-phan
	%		%DM	%DM	%DM	%	%DM	%DM	%DM	%DM	%DM	%DM	%DM
WD01	14.2	0.59	4.3	12.5	16.2	83.257	3	1.9					
WD02	9.4	1.83	18.2	46.1	18.5	16.298	7.5	5.1	0.00	0.49	0.55	0.91	0.00
WD03	12.3	0.47	3	8	15.8	84.956	3.2	1.7	0.00	0.34	0.49	0.64	0.00
WD04	68.4	1.38	9.7	23.8	26.9		7.4	3.5	0.00	0.63	1.30	1.36	0.00
WD05	94.6				90.7		0	13	0.00	0.00	1.67	1.67	13.15
WB01	14.2	0.72	4.4	11.2	15.7	89.3	2.8	2.6					
WB02	9.2	3.46	25	54.1	13.7	14.437	5.4	7.6	0.00	0.35	0.61	0.85	0.00
WB03	12.5	0.65	6.4	7	15.7	72.539	2.9	1.7	0.00	0.34	0.53	0.66	0.00
WB04	76.5	2.49	13.7	32.5	34.6		10.8	3.8	0.00	0.85	1.62	1.74	0.00
WB05	93.3				83.6		0	10.4	0.00	0.00	1.49	1.79	10.90
BM01	12.7	1.18	8.7	21.8	11.9	63.979	3.7	5.2					
BM02	8.1	2.33	29.1	52	14.3	12.162	6.1	9	0.00	3.18	0.88	0.74	0.00
BM03	11.4	0.37	4.2	10.4	11.7	65.028	2.4	1.7	0.00	0.24	0.44	0.52	0.00
BM04	70.1	0.84	12.2	24.8	37.5		7.2	3.3	0.00	7.83	2.14	1.61	0.00
BM05	93.8				77.4		0	14.5	0.00	0.00	0.81	1.61	11.94
BH01	11.2	1.46	11.4	27.5	14.4	58.903	3.5	3.9					
BH02	7.3	2.32	22.5	48.8	13.6	19.402	4.7	6.9					
BH03	10.9	0.55	3.8	10.7	13.5	67.795	2.9	2	0.00	0.30	0.72	0.71	0.00
BH04	68.3	1.72	10	23.8	35.8		5.9	3.2	0.00	0.79	1.42	1.67	0.00
BH05	94.2				82.8		0	13.8	0.00	0.00	2.24	1.90	18.45
PY01	13.1	0.07	11	13.6	20.8	47.178	2.1	2.8					
PY02	7.7	2.02	64.1	72.9	5.5	1.863	1.2	2.8	0.00	0.13	0.08	0.21	0.74
PY03	10.8	0.08	4.8	10.1	22.6	61.836	2.2	2.7	0.00	0.59	0.17	0.81	0.72
PY04	66.6	0.34	7	15.5	32.2		6.4	5.5	0.00	0.78	0.09	1.02	1.59
PY05	78.7				26.3		58.7	6.1	0.00	0.52	0.28	0.66	3.62
PG01	10.9	0.09	9.7	13	22.7	48.604	2.4	2.5					
PG02	7.5	2.58	60	67.8	6.2	4.166	1.2	2.6					
PG03	11.1	0	4.2	13.6	26.1	53.117	2.2	2.7	0.00	0.69	0.10	0.88	0.54
PG04	66.4	0.29	11	12	35		3.5	5	0.00	1.07	0.21	1.40	1.31
PG05	91				75.6		3.3	17.8	0.00	2.11	0.78	1.89	11.78
O1V01	12.9	1.7	13.3	24.6	9.3	38.036	7.9	3.2					
O1V02	9.8	5.43	43.2	78.6	3.8	6.208	2.7	6.3	0.00		0.09	0.12	0.88
O1V03	10.9	1.08	4.7	14.6	11.7	67.436	8	2.4	0.00	0.20	0.13	0.36	0.58
O1V04	71	3.44	13.2	29	30.4		27.5	5.5	0.00	0.48	0.21	0.93	2.21
O1V05	91.6				66.7		25	17.9	0.00	0.00	0.83	1.43	11.07
O2V01	12.1	1.73	12.2	24.3	14.1	30.969	7.6	2.8					
O2V02	8.6	5.55	44	79.3	3.5	2.736	2.6	6					
O2V03	11.1	1.35	6.7	21.1	16.6	53.3	7.9	2.4	0.00	0.26	0.19	0.55	0.76
O2V04	68.9	3.39	15.5	31.9	28.6		27.1	5.2	0.00	0.35	0.16	0.74	1.77
O2V05	91.2				63.6		26.1	17	0.00	0.00	0.80	1.36	10.80
T01	12.3	0.82	4.6	15.1	16.2	35.795	3.1	2.2					
T02	9.4	1.92	11	33.2	19.3	20.606	5.5	4.3	0.00	0.43	1.04	1.03	0.00
T03	11.8	0.74	2.5	10.2	22.1	64.699	2.3	1.8	0.00	0.36	0.61	0.69	0.00
T04	75.6	2.67	12.3	35.1	35.5		8.5	3.8	0.00	8.77	2.01	1.60	0.00
T05	92.2				75.6		24.4	11.5	0.00	0.00	0.77	1.54	10.26
WCPS01	15	0.68	4	11.4	14.6	73.436	2.8	1.6					
WCPS02	11.3	2.29	12.6	33.7	17	30.181	5.3	4.2	0.00	0.20	0.19	0.59	1.17
WCPS03	13.5	0.52	2.8	8.3	14.6	78.154	2.5	1.6	0.00	0.12	0.13	0.37	0.66
WCPS04	68.8	2.56	11.8	28.8	29.4		7	5.4	0.00	0.45	0.16	0.99	2.12
WCPS05	92.7				74		16.4	16.4	0.00	0.00	1.10	1.51	14.93

Note the high water content values for the thin stillage (05) fractions. The liquid-liquid extraction process means that this material is essentially water with some soluble material. The other analysis results (Protein, Fat, and Ash) are still given in %DM (Dry Matter), however, meaning that the content in the overall stream is significantly lower.

Figure 3: Bran and Hulls Fibre and Protein

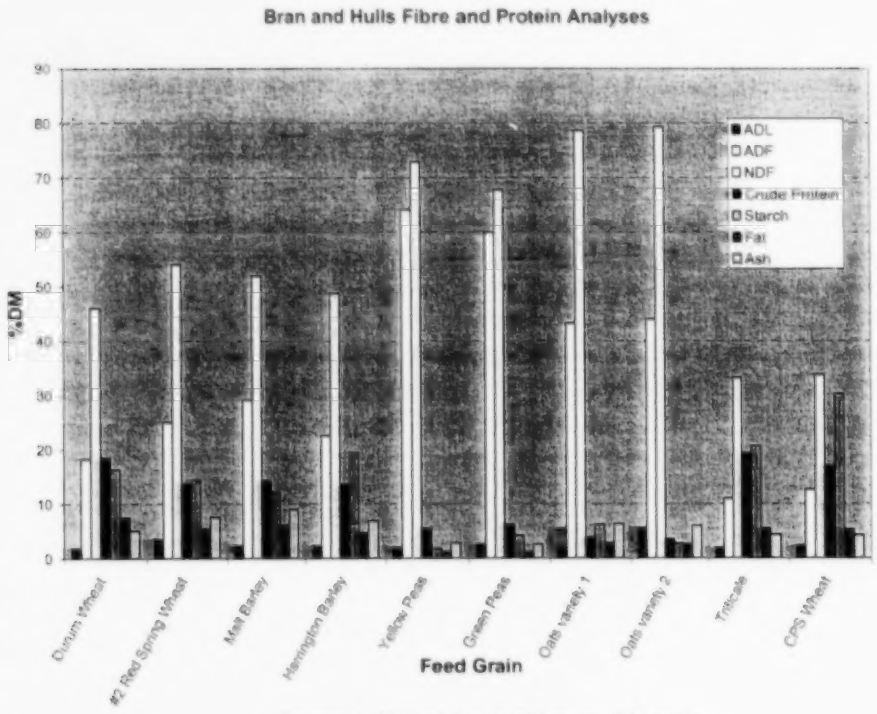


Figure 4: Milled Grain Fibre and Protein

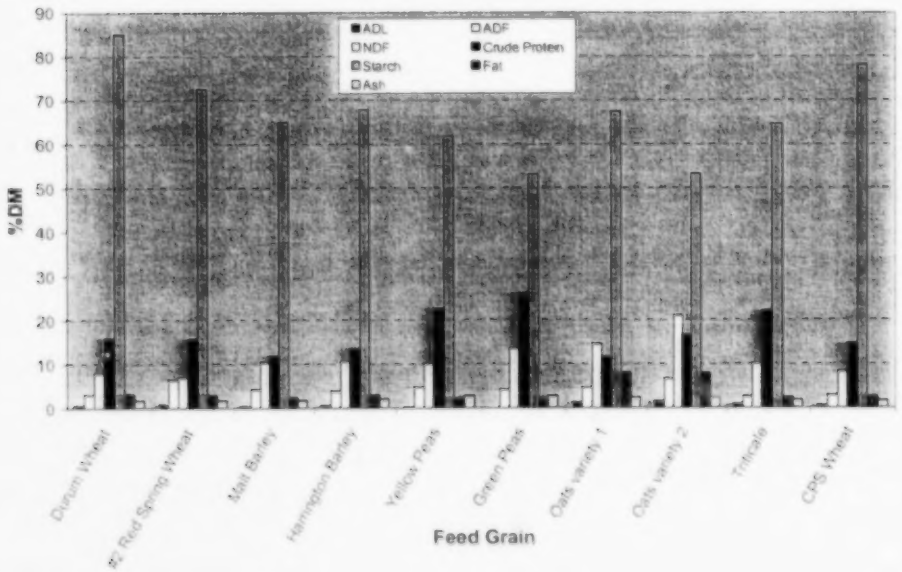


Figure 5: Milled Grain Amino Acids

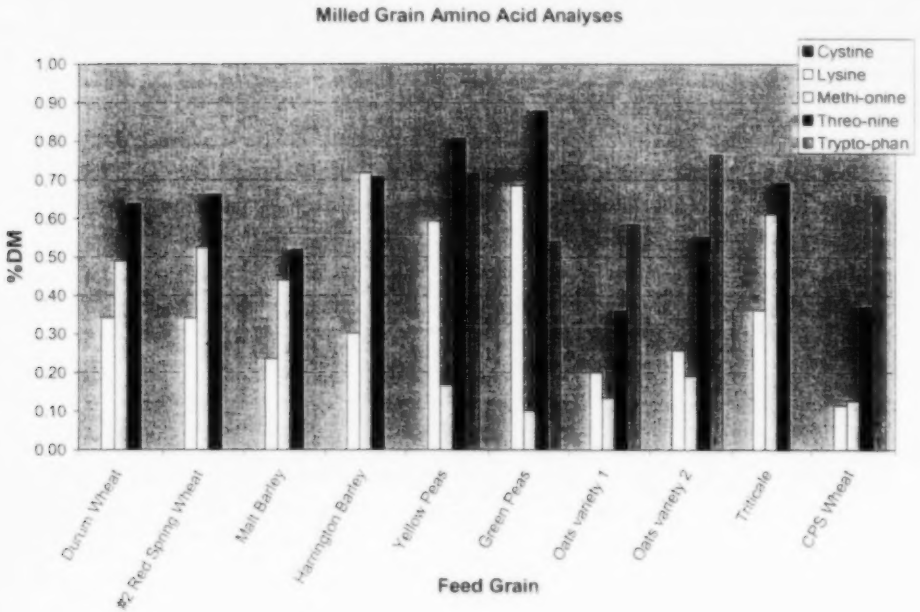


Figure 6: Fermenter Solids Fibre and Protein

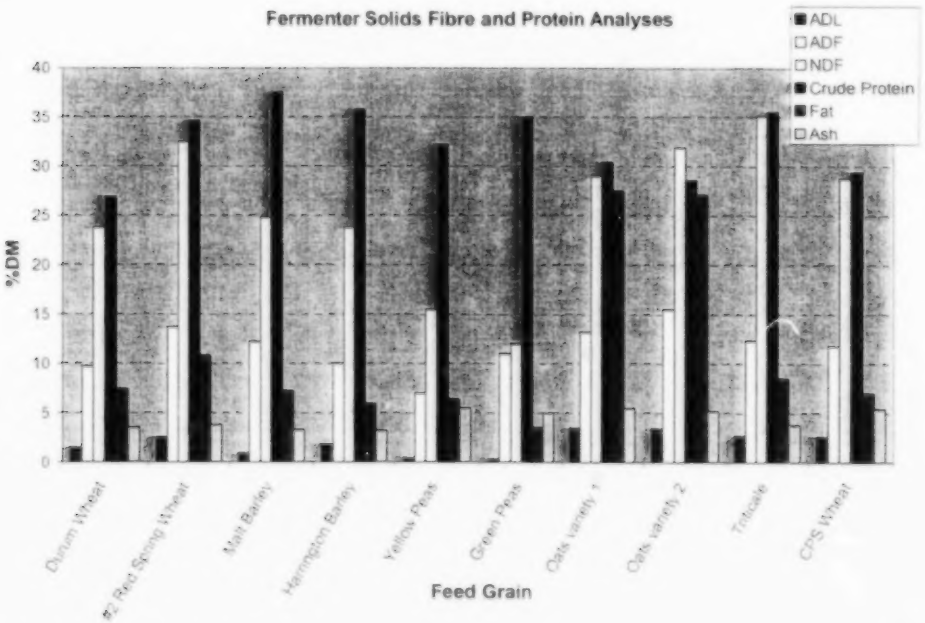


Figure 7: Fermenter Solids Amino Acids

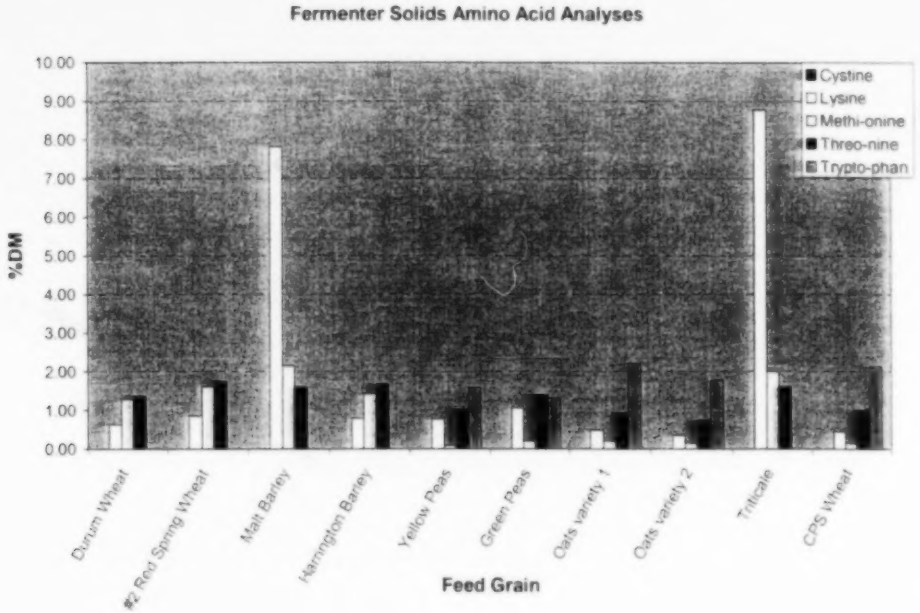


Figure 8: Thin Stillage Fibre and Protein

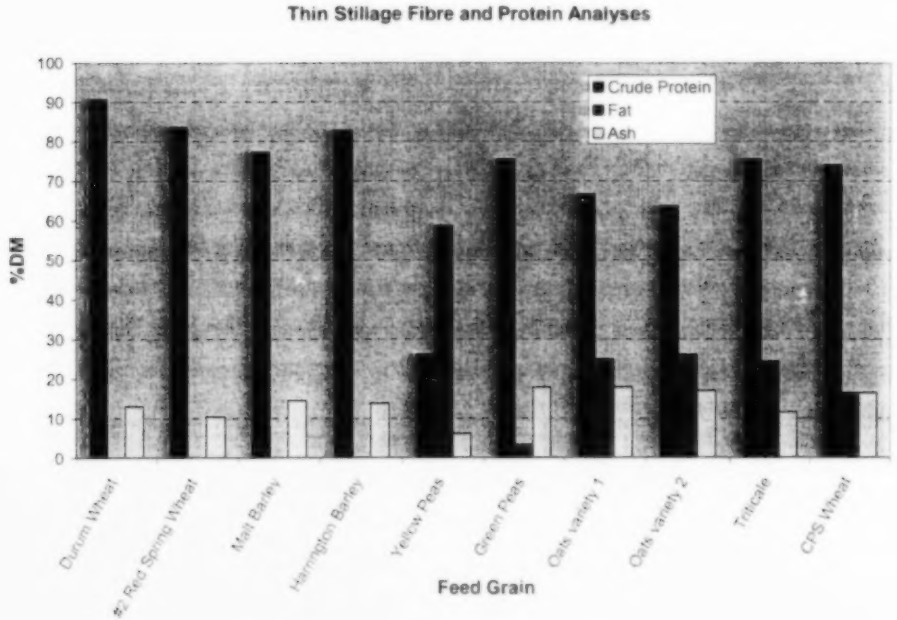
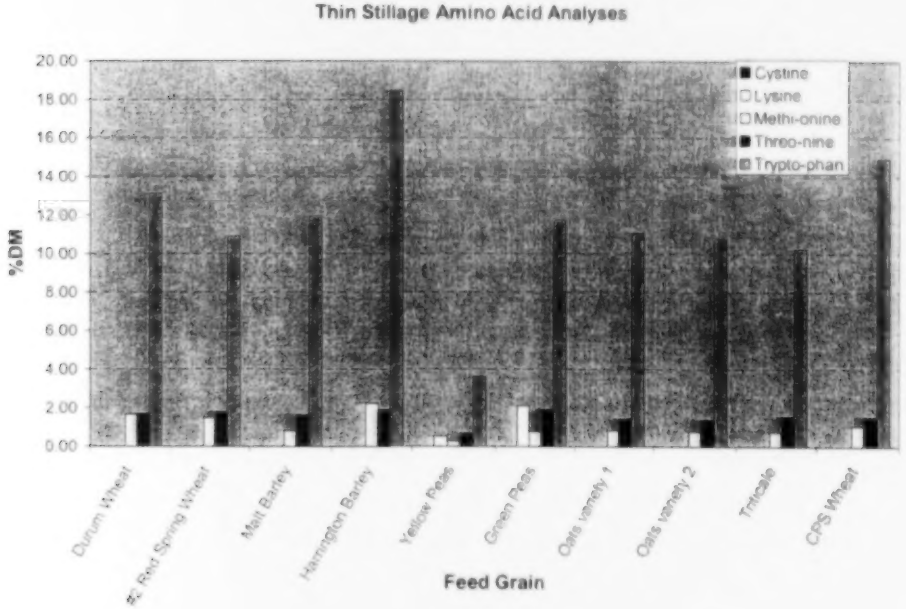


Figure 9: Thin Stillage Amino Acids



Discussion

Starch Distribution

Starch analyses were done on the milled grain that was added to the fermenters, the raw grain, and the bran and fines (or hulls in the case of the peas). As expected, the starch content was highest in the milled grain, followed by the raw grain. Durum and CPS wheat had the greatest amount of starch in the raw grain. However, once milled, most of the grains had in excess of 60% starch, except for the second oats variety and the green peas (see Figure 4). The bran and fines contained only small amounts of starch (See Figure 3), and milling of the grain to remove these components increased the starch content of the flour by 2 to 29% above the starch content in the raw grains.

Note that the starch content indicated for the raw grain for the triticale is significantly lower than that for the other similar grains (e.g. wheat). It is suspected that this value is in error. The starch analysis is a difficult one, and even though the procedure to be used was validated by an independent party, it is not uncommon to see inaccurate results.

Starch analyses were also done on some of the fermenter solids, but these were inconclusive. It is suspected that interference with the assay resulted in the percentages of starch in the fermenter solids being questionable. It is expected that the majority of starch would have been hydrolyzed and the glucose fermented during the fermentation process.

Nutrition Content

Wheat Derivatives (Durum, Red Spring, Canada Prairie Spring, Triticale):

These grains have protein contents and carbohydrate fractions that are typical (see Figure 2). As expected, the bran and fines resulting from de-branning are high in neutral detergent fibre (NDF) with only moderate amounts of acid detergent lignin (ADL) indicating that a reasonable digestibility coefficient could be expected in ruminants. (See Figure 3) This fraction readily lends itself for use as a soluble fibre source in animal diets or if refined, in human diets.

The results for the fermentation co-products lead to some interesting conclusions. Amino acid levels of fermenter solids in most cases in this project are not consistent with what would be expected, particularly for lysine. In the wheat derivatives, for example, the lysine content of the raw grain would normally average 0.4%. However, the milled wheat shows lysine levels less than this (See Figure 7). This is also true for the other amino acids indicating that a larger portion of these amino acids are likely resident in the bran and fines portion of the wheat. In the thin stillage, the high protein content offers potential as use as a high protein fraction (See Figure 8).

These results indicate that milling of the wheat derivatives to remove the bran leaves lower protein fermenter solids, which may actually have greater use in diets for ruminants. The lower protein however limits use of the fermenter solids for monogastrics. The high protein content of the thin stillage has potential as has the bran and fines fraction, which would be valued for its fibre content.

Barley (Malt and thin Harrington):

The barley results show a similar trend to that of the wheat, but with higher protein yields in the fermenter solids (See Figure 6). The thin stillage protein contents are high but like that found in the wheat, amino acid levels in the fermenter solids are lower than expected (See Figure 7). Sample preparation or essential amino acid use by the strain of microbes used in the fermentation could be the reason for this discrepancy.

Although these experimental results indicate that the fibre content of the barley bran fractions is very similar to that of wheat (See Figure 3), previous experience indicates that this is normally not the case. Typically, the high indigestible fibre content of the barley bran fraction makes it of limited use, and de-branning of barley must be based on increased efficiency of fermentation for justification.

Peas (Yellow and Green):

The hull and fibre fractions for peas are well known for their digestible soluble fibre and have use in feed or food. This is reflected in the results from this testing program (See Figure 3). The higher proteins resulting from de-hulling peas did not necessarily translate into higher proteins in the fermenter solids (See Figure 4 and Figure 6). The high protein content of the thin stillage offers potential as a quality protein source. Further with peas, pre-processing by air classification to strip protein and the manufacture of a pea protein concentrate destined for aquaculture has promise. Peas hold potential as a valued protein source by pre-processing and also by the high protein thin stillage fraction resulting from the process.

Oats:

The bran and fines fractions resulting from oats are high in NDF but are higher in ADL than the other grains (See Figure 3). This high level of ADL would substantially affect digestibility in a negative manner. Thus, the value of the hull and fines fraction would be limited. The fermenter solids and thin stillage fractions from oats are not only high in protein but in fat as well, especially the fermenter solids. This provides two co-products with unique characteristics that can have value in feeding (i.e., high fat and protein).

Anomalous Readings:

Note that there are some readings that are significantly different than expected for no apparent reason (e.g. the high spikes in lysine for Malt Barley and Triticale in Figure 7, the high fat value in yellow peas in Figure 8, and the low tryptophan value for peas in Figure 9). These are acknowledged but there is no explanation for the anomalies.

It was also noted that the results for tryptophan in the fibres, milled grain, and fermenter solids for the first five grains tested (Durum and Red Spring Wheat, Malt and Harrington Barley, and Triticale) all indicated undetectable, while those for the other grains had positive results. It is suspected that this is due to a delay in the testing for tryptophan for the first batch of samples. Some degradation of the materials may have occurred, resulting in a loss of tryptophan content.

Conclusions and Recommendations

Significant Conclusions based on project objectives

The premilling step was successful in increasing the starch content of all of the grains tested, at the same time generating a low-starch fibre stream. The protein split through premilling was fairly even for the wheat, barley and triticale. For the peas and oats, the proteins tended to preferentially end up in the milled grain rather than the fibre stream. This step did provide some of the fractionation that is necessary to allow more appropriate use of each of the components of the raw grain, particularly with wheat, peas, and wheat-related materials such as triticale.

As expected, there was a significant increase in the concentration of the proteins in the fermenter solids and thin stillage compared to the milled grain. The effect was less pronounced for the peas than expected, as the coproducts from peas were similar in nutritional value to those from the other grains. Also, this is not always translated into higher amino acids.

The fermentation and processing methods used in this project have created markedly different co-products from ethanol production than those from traditional processes, creating new opportunities for designer feeds. For example, the high fat content in oat fermenter solids and thin stillage coupled with the high protein level is a unique opportunity combination.

The high protein content of all of the resulting thin stillage materials offers potential for use as a high protein ingredient in young animal and aquaculture feeds.

There is value in different forms in all of the prairie grains tested (and likely in many others as well). Wheat and its derivatives, barley, and peas are the most promising sources of feedstock for a combined ethanol and animal feed plant. Although peas are not typically used for this purpose, they can be processed using the same equipment as wheat and barley, and the resultant pea meat is highly nutritious. Oats in their whole form necessitate some additional milling steps as compared to other cereal grains, and tend to have a shorter shelf life due to the higher oil content.

Recommendations for application of results

Premilling should be incorporated into an industrial process to facilitate the creation of designer feeds suited for specific purposes (animal or ethanol process feed). Even the fibrous streams from different grains have unique characteristics. Most cereal grains can be processed using similar equipment to maximize flexibility of the facility. The inclusion of the ability to process oats, however, requires consideration of the added cost for the requisite extra equipment relative to the added value of the co-products.

It should be noted that preliminary milling for bran removal is most similar to barley pearling in terms of existing milling technology. Therefore, the milling equipment for the purposes of fibre removal for ethanol feedstock and animal feed should ideally be sourced from the same manufacturers that produce the equipment used in modern barley pearling plants.

A preliminary milling plant for these purposes should incorporate multiple milling steps for optimal flexibility of fibre removal. With an adequate combination of frictional and abrasive milling steps and control over the fibre removal process designed into a preliminary milling plant, it will be possible to debran various grains by varying degrees for different usages.

In a commercial operation, monitoring and analysis must be done on a continual basis to ensure proper marketing and blending of co-products, due to the different nutritional results for the co-products from different grains.

Recommendations for future work

Further experimentation with different premilling technologies and process tuning could be done to optimize the separation of fibre and starch. This could likely be done at the pilot plant scale to make the conclusions more relevant.

It may be beneficial to complete amino acid analysis on the raw grains and / or the bran and fibres streams to confirm the apparent conclusion that these components migrate towards the fibre rather than the starch.

Acknowledgements

The following personnel made significant contributions to the information in this report:

March Consulting Associates Inc.

Jason Gogal – Junior Process Engineer

Dongmei Fang – Mid Level Process Engineer

Tara Zrymiak – Senior Process Engineer

Prairie Feed Resource Centre

Vern Racz – Executive Director

ALS Laboratory Group (formerly known as Enviro-Test Labs)

Raechelle Kreese – Account Manager

Saskatchewan Research Council

Dr. Malcolm Rollins – Manager, Fermentation Technologies

Amy Friesen – Research Scientist, Fermentation Technologies

Robert Ashton – Technologist, Fermentation Technologies

And other laboratory personnel at SRC

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Literature Cited

Starch Analysis Procedure:

"Megazyme Starch Procedure.pdf"

Amino Acid Analysis Procedure:

"GMP4_16.02 Amino Acid SOP Summary.pdf"

Appendix A – Nutrition Results Charts by Grain

Figure 10: Durum Wheat Fibre and Protein

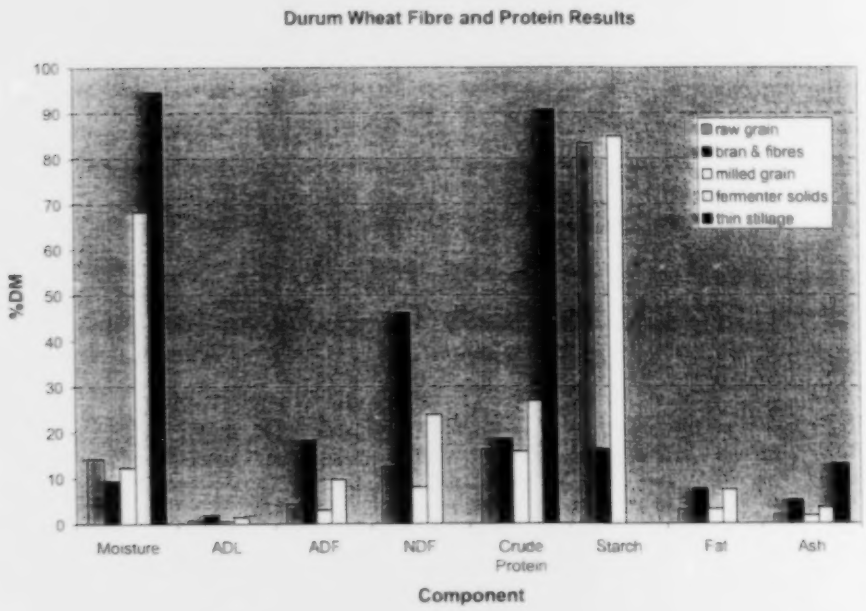


Figure 11: Durum Wheat Amino Acids

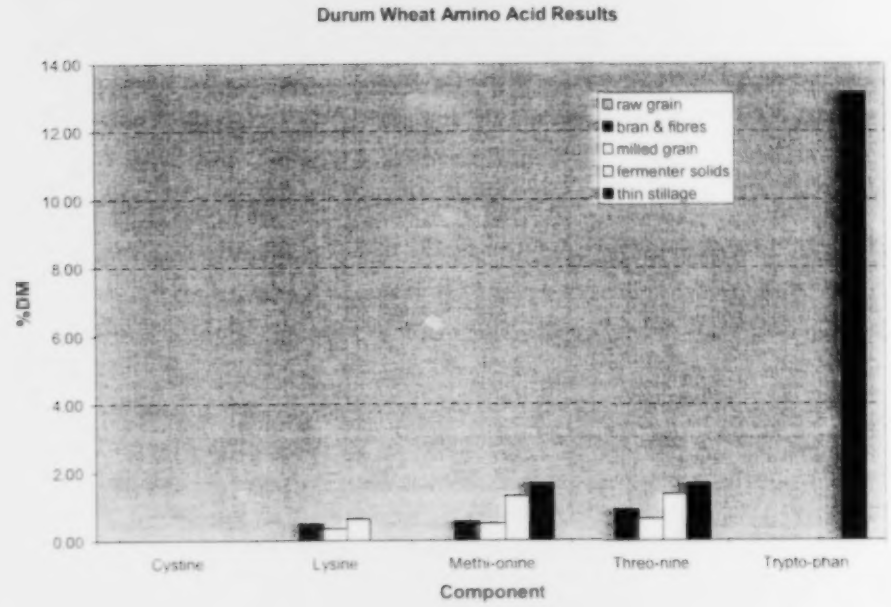


Figure 12: #2 Red Spring Wheat Fibre and Protein

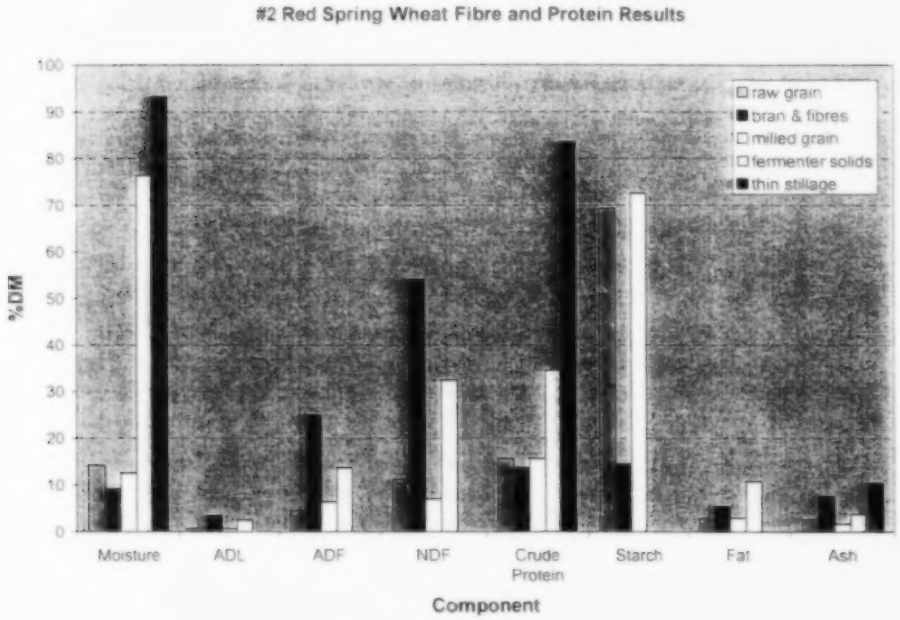


Figure 13: #2 Red Spring Wheat Amino Acids

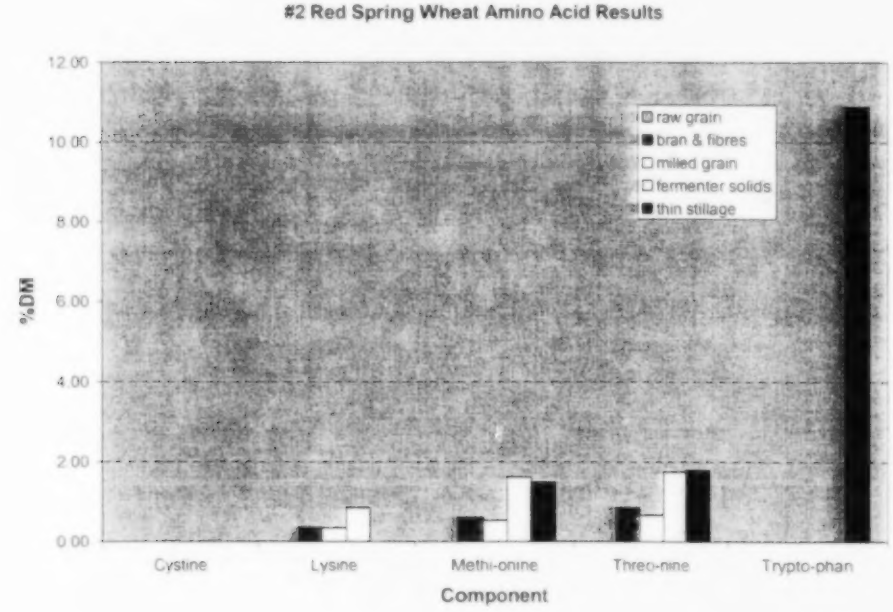


Figure 14: Malt Barley Fibre and Protein

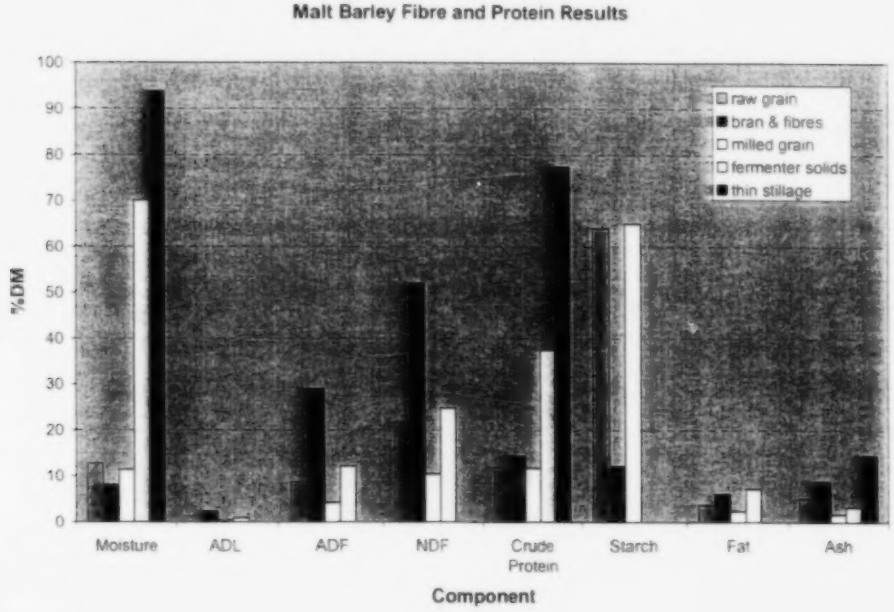


Figure 15: Malt Barley Amino Acids

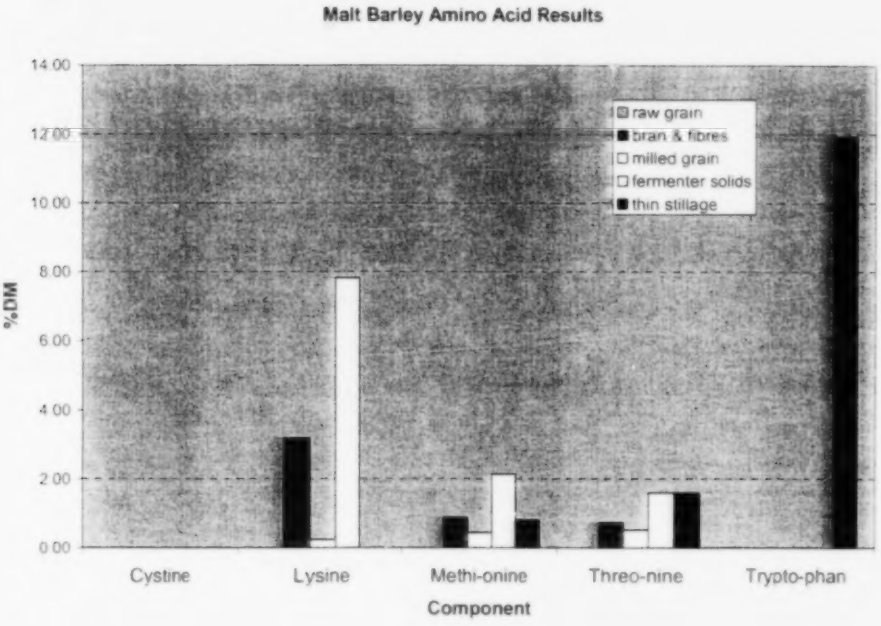


Figure 16: Harrington Barley Fibre and Protein

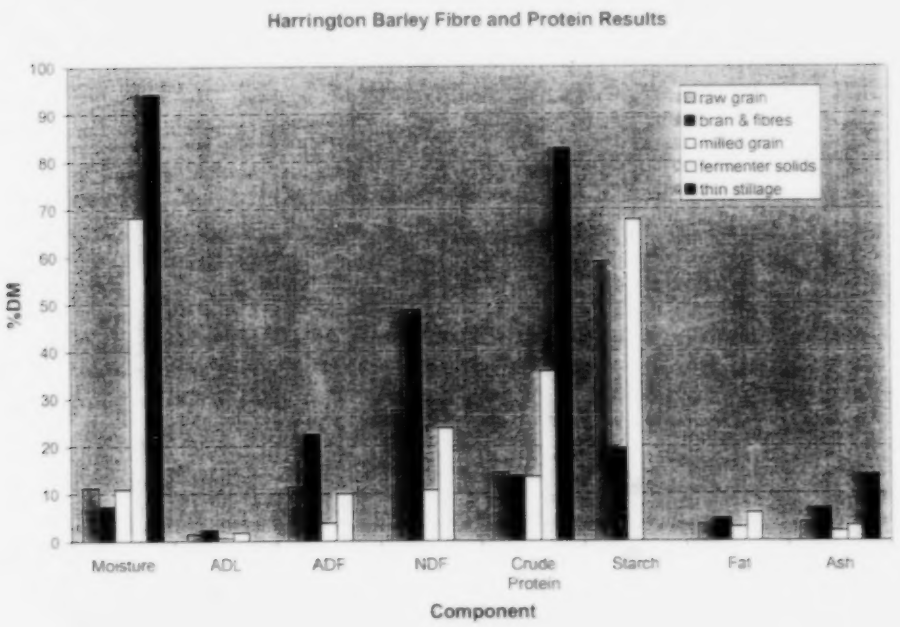


Figure 17: Harrington Barley Amino Acids

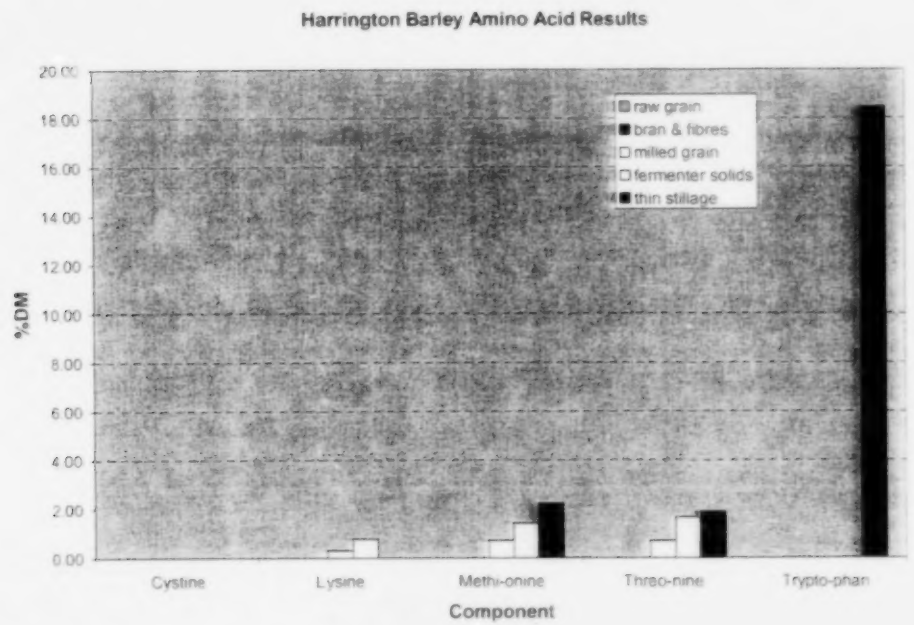


Figure 18: Yellow Peas Fibre and Protein

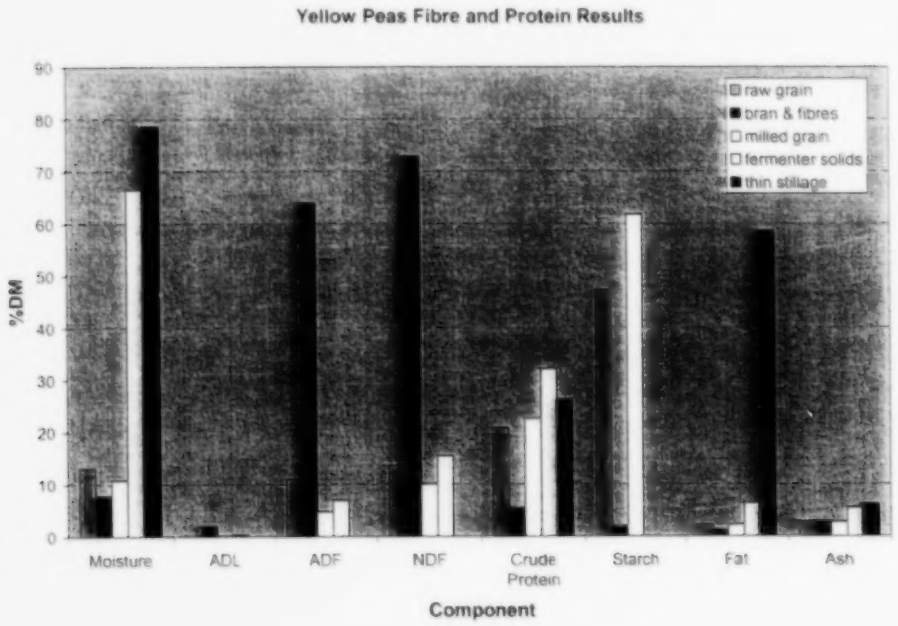


Figure 19: Yellow Peas Amino Acids

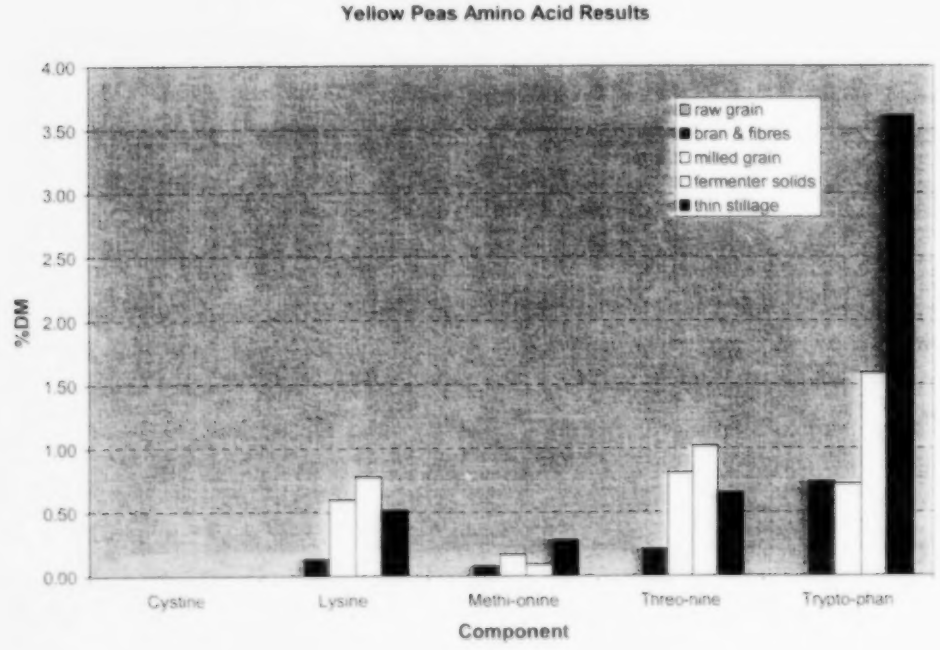


Figure 20: Green Peas Fibre and Protein

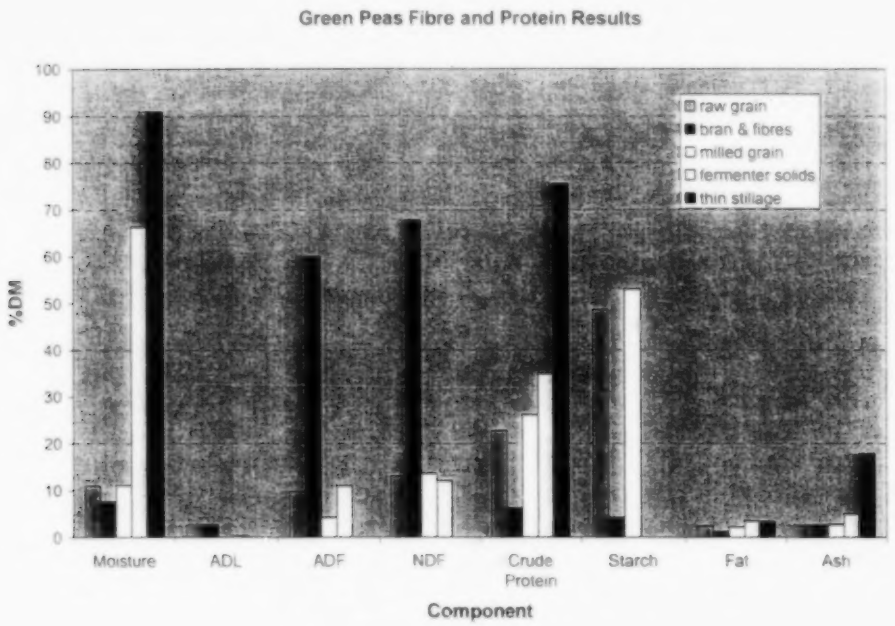


Figure 21: Green Peas Amino Acids

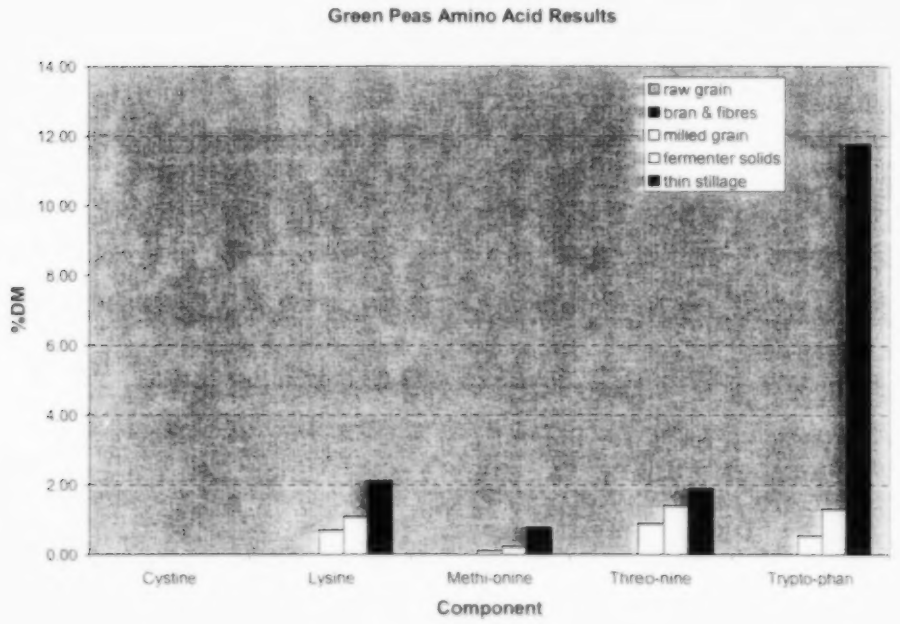


Figure 22: Oats Variety 1 Fibre and Protein

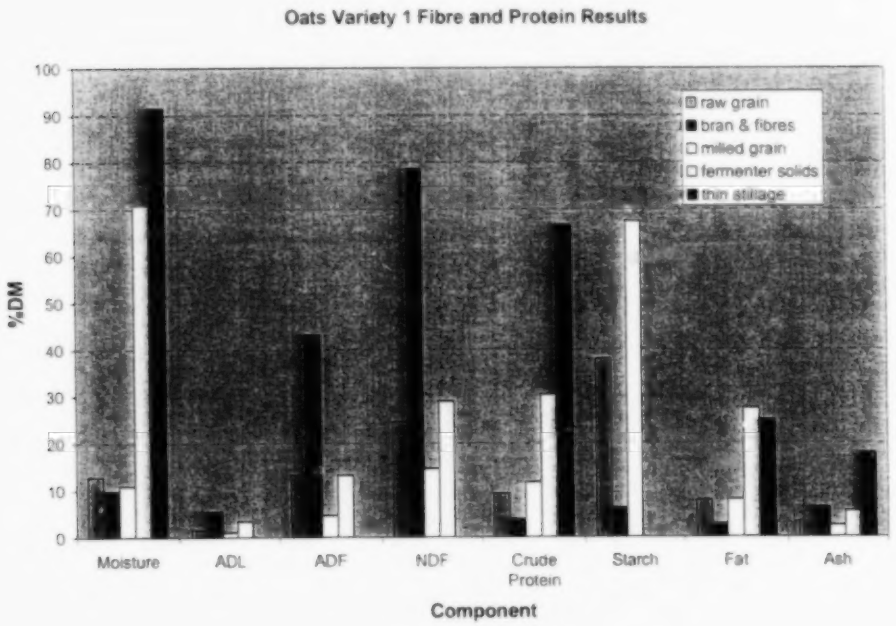


Figure 23: Oats Variety 1 Amino Acids

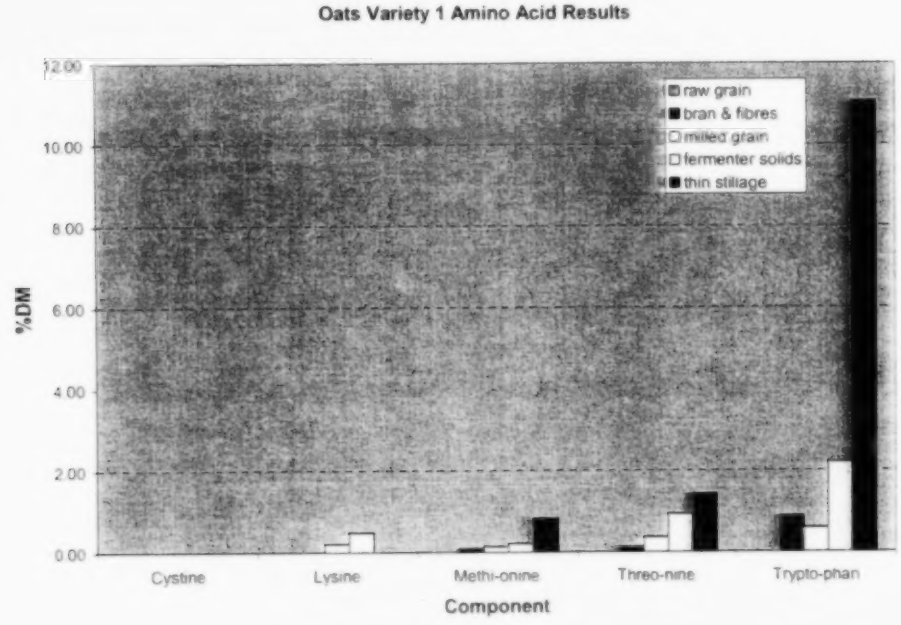


Figure 24: Oats Variety 2 Fibre and Protein

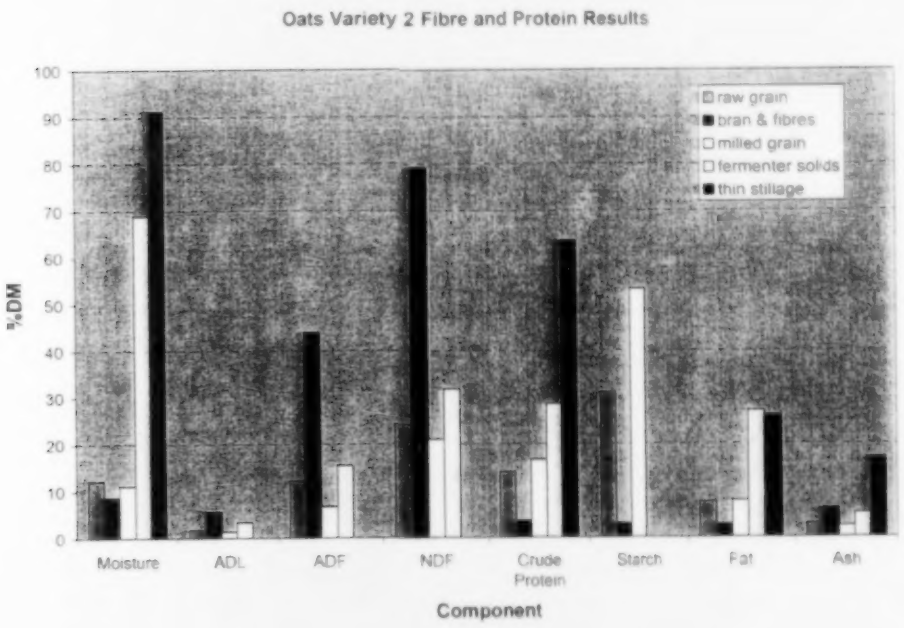


Figure 25: Oats Variety 2 Amino Acids

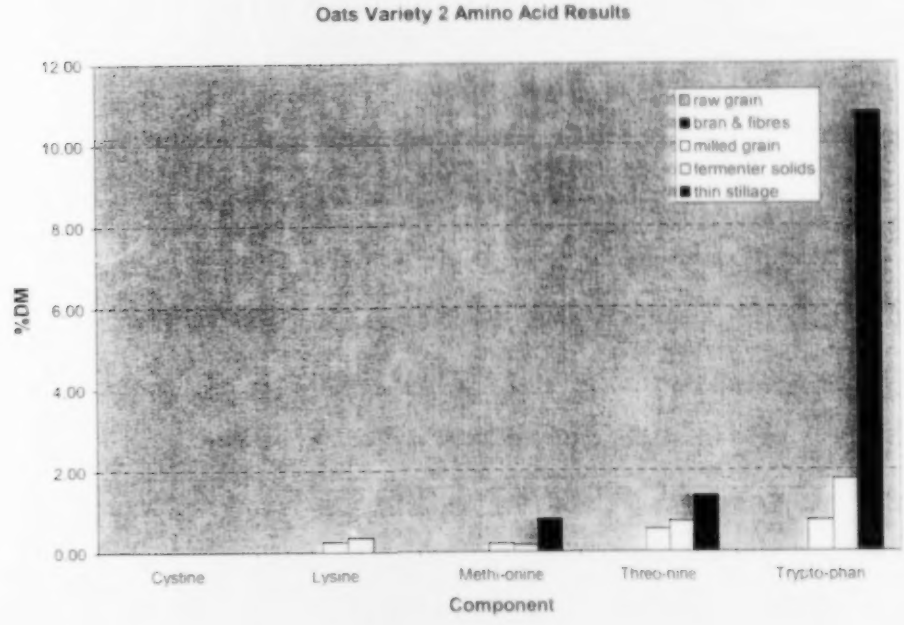


Figure 26: Triticale Fibre and Protein

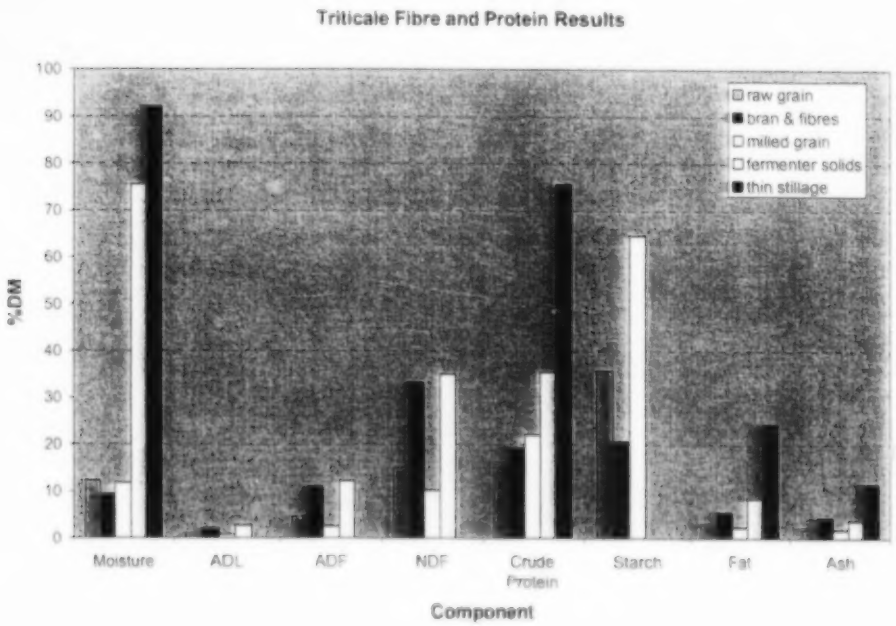


Figure 27: Triticale Amino Acids

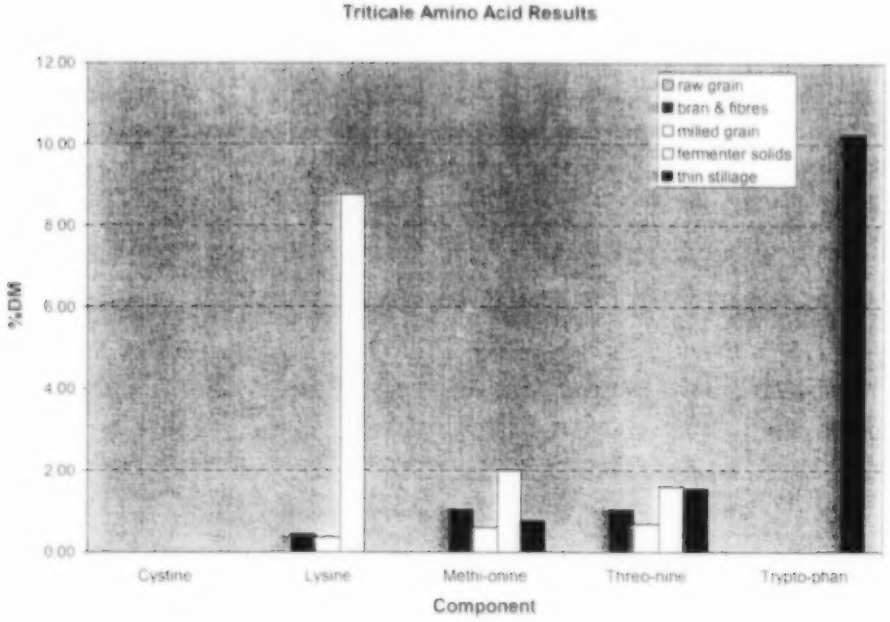


Figure 28: CPS Wheat Fibre and Protein

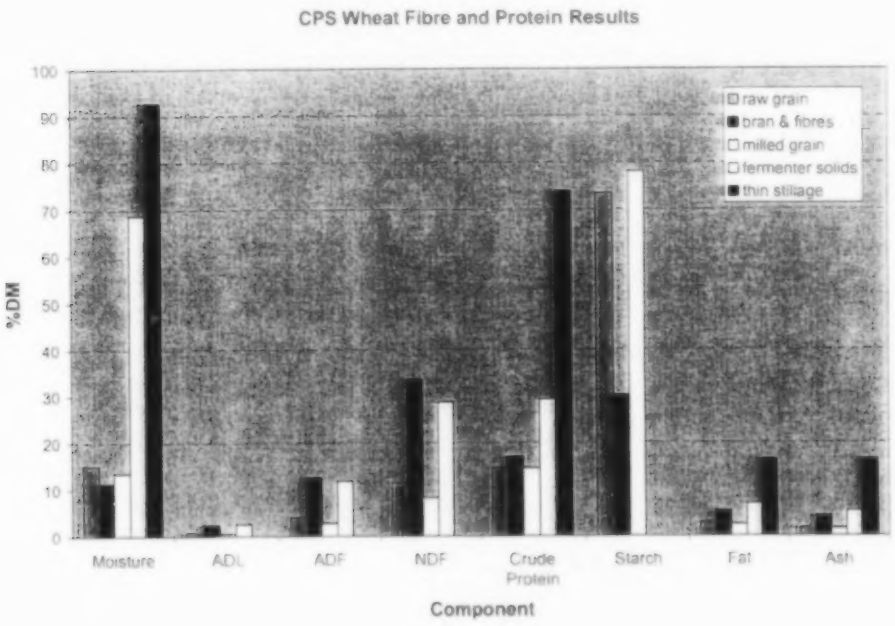
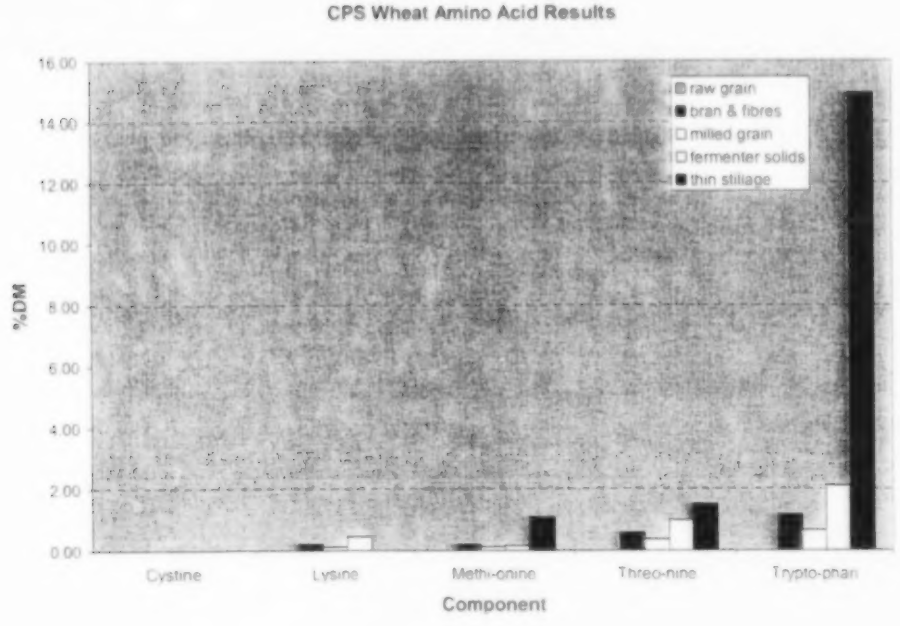


Figure 29: CPS Wheat Amino Acids



PRINCIPLE:

The Megazyme total starch analysis procedure (AA/AMG) allows the measurement of total starch in most cereal products (natural or processed). In the assay format described, starch hydrolysis proceeds in two phases. In phase I, starch is partially hydrolysed and totally solubilised. In phase II, the starch dextrins are quantitatively hydrolysed to glucose by amyloglucosidase.

For most samples (e.g. wheat flour), complete solubilisation of starch can be achieved by cooking the sample in the presence of thermostable α-amylase. However, for samples containing high levels of resistant starch (e.g. high amylose maize starch), complete solubilisation and dextrinisation requires pre-treatment with dimethyl sulphoxide (DMSO) at 100°C.

Samples containing high levels of glucose and maltodextrins are washed with aqueous ethanol before analysis.

Analysis of a single sample can be performed within 70 min. Twenty samples can be analysed within 2 hr.

ACCURACY:

Standard errors of ±2% are achieved routinely.

KITS:

Kits contain sufficient reagent for performing 100 assays i.e. they contain the full assay method plus:

1. Thermostable α-amylase (10 mL, 3,000 Units/mL)
   Store at 4°C. Stable for >3 years.
2. Amyloglucosidase (10 mL, 200 Units/mL)
   Store at 4°C. Stable for >3 years.
3. Glucose Determination Reagent (GOPOD; high purity)
   Store dry at 4°C. Long term storage at -20°C.
4. Glucose Reagent Buffer (concentrate)
   Store at 4°C.
5. Glucose Standard Solution
   Store at room temperature.
6. Regular Maize Starch
   Store at room temperature.

REAGENTS:

1. **MOPS buffer** (50 mM, pH 7.0) plus calcium chloride (5 mM) and sodium azide (0.02%).

   - MOPS (sodium salt, 11.55g; Sigma Chemical Co.; cat. no. M9381) is added to 900 mL of distilled water. This solution is adjusted to pH 7.0 by the addition of 1 M (10%) HCl. Approximately 17 mL is required.
   - Calcium chloride dihydrate (0.74 g) and sodium azide (0.2 g) is added and dissolved. The volume is adjusted to 1 litre and the buffer is stored at room temperature.

2. **Sodium acetate buffer** (200 mM, pH 4.5) plus sodium azide (0.02%).

   - Glacial acetic acid (11.8 mL, 1.05 g/mL) is added to 900 mL of distilled water. This solution is adjusted to pH 4.5 by the addition of 1 M (4 g/100 mL) sodium hydroxide solution. Approximately 60 mL is required.
   - Sodium azide (0.2 g) is added and the volume is adjusted to 1 litre. Store at room temperature.

NOTE

Sodium azide should not be added until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.

3. **Dimethyl sulphoxide** (DMSO) (laboratory grade). Available from most chemical suppliers including Sigma Chemical Co. (cat. no. D5879).

NOTE

Dimethyl sulphoxide is listed in the Merck Index (No. 3255) as a skin irritant and thus, it should be used with caution.

Sodium azide is a toxic chemical and should be treated accordingly. It is added to buffers solely as a preservative. It can be deleted from buffer recipes, but buffers should then be stored at 4°C.

SAMPLE BLANKS:

Sample blanks can be determined using the Standard Assay Procedure format with the modifications that in Step 4, three (3) mL of distilled water is used and in Step 5, amyloglucosidase is replaced by water. Alternatively, the need to perform sample blank analysis can be avoided by pre-extraction of samples with aqueous ethanol (refer below).

A. STANDARD ASSAY PROCEDURE (AA/AMG)

1. Mill cereal product to pass a 0.5 mm screen.
2. Add flour sample (~100 mg; weighed accurately) to a glass tube (16x120 mm). Tap the tube to ensure that all of the sample drops to the bottom of the tube.
3. Wet with **0.2 mL of aqueous ethanol** (80% v/v) to aid dispersion, and stir the tube on a vortex mixer.
4. **Immediately** add **3 mL of thermostable α-amylase** (300 Units) in MOPS buffer (50 mM, pH 7.0) and **vigorously stir** the tube on a vortex mixer. **Incubate** the tube in a boiling water bath for **6 min**. (Stir the tube vigorously after 2 min and 4 min.)

NOTE

In this step it is essential that the tube is stirred vigorously to ensure complete homogeneity of the slurry (removal of lumps). Also, stirring after 2 min prevents the possibility of some of the sample expelling from the top of the tube when the alcohol is evaporating.

5. Place the tube in a bath at 50°C; add **sodium acetate buffer (4 mL**, 200 mM, pH 4.5), followed by **amyloglucosidase (0.1 mL, 20 U)**. Stir the tube on a vortex mixer and incubate it at 50°C for 30 min.
6. Transfer the entire contents of the test tube to a 100 mL volumetric flask. Use a wash bottle to rinse the tube contents thoroughly. Adjust to volume with distilled water. Mix thoroughly. Centrifuge an aliquot of this solution at 3,000 rpm for 10 min.

ALTERNATIVELY, at Step 6, adjust the volume to 10 mL with distilled water and then centrifuge the tubes at 3,000 rpm for 10 min. For samples containing 1-10% starch content, this solution is used directly in Step 7. For samples containing 10-100% starch, an aliquot (1.0 mL) is diluted to 10 mL with distilled water before proceeding to Step 7.

5. Proceed from **Step 4** of the Standard Assay Procedure (AA/AMG).

   Alternatively:

   Proceed from **Step d** of the Modified Assay Procedure (DMSO/AA/AMG) if **DMSO** treatment is required.

CALCULATIONS:

$$\text{Starch} = \Delta E \times F \times 1000 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta E \times \frac{F}{W} \times 90$$

where:

ΔE = Absorbance (reaction) read against the reagent blank.

F = $\dfrac{\textbf{100 (µg of glucose)}}{\textbf{absorbance of 100 µg of glucose}}$ (conversion from absorbance to µg)

1000 = Volume correction (0.1 mL taken from 100 mL).

$\frac{1}{1000}$ = Conversion from micrograms to milligrams.

$\frac{100}{W}$ = Factor to express "starch" as a percentage of flour weight.

W = The weight in milligrams ("as is" basis) of the flour analysed.

$\frac{162}{180}$ = Adjustment from free glucose to anhydro glucose (as occurs in starch).

Starch % (dry wt. basis):

$$\textbf{= Starch \% (as is)} \times \frac{100}{\textbf{100 - moisture content (\%)}}$$

Table 1. Results of an interlaboratory evaluation of the total starch assay procedure (Procedure A and Procedure B)

Sample	Chicken Feed Pellets	White Bread	Green Pea	High Amylose Maize Starch*	White Wheat Flour
Moisture %	11.4	10.7	12.4	13.4	12.8
No. of labs	32	32	31	25	31
Outliers	0	0	1[G]	1[C]	1[C]
Average %	50.7	68.1	44.0	86.3	78.0
S_r	1.6	1.8	1.5	2.5	2.2
RSD_r	3.1	2.7	3.4	2.9	2.9
r	4.4	5.2	4.2	7.0	6.3
S_R	2.4	3.4	2.1	4.1	3.3
RSD_R	4.6	5.0	4.8	4.8	4.2
R	6.6	9.5	6.0	11.6	9.2
HORRAT	2.1	2.4	2.1	2.8	2.0
Range	45.4-55.3	62.0-74.9	39.4-47.4	78.7-96.8	71.6-85.8

Table 1. (cont.)

Sample	Wheat Starch*	Oat Bran	Spaghetti	High Amylose Maize Starch DMSO procedure	Wheat Starch DMSO procedure
Moisture %	12.3	8.8	11.8	13.4	12.3
No. of labs	26	31	31	31	31
Outliers	0	1[C]	1[C]	1[C]	1[C]
Average %	97.2	42.2	76.6	97.2	96.5
S_r	3.2	1.6	3.0	2.0	3.0
RSD_r	3.3	3.8	3.9	2.1	3.1
r	9.0	4.5	8.4	5.7	8.4
S_R	3.7	2.1	3.7	2.8	4.4
RSD_R	3.8	5.0	4.8	2.9	4.6
R	10.4	6.0	10.3	7.8	12.4
HORRAT	1.9	2.2	2.3	1.4	2.3
Range	91.8-105.0	38.7-46.8	70.1-81.8	91.6-101.9	86.0-104.0

Number of Labs = Number of laboratories included in calculations
Outliers = Number of outlier laboratories, not included in calculations ([C]=Cochran, [G]=Grubbs outlier)
S_r = Repeatability standard deviation
RSD_r = Repeatability relative standard deviation
r = Repeatability value ($2.8 \times S_r$)
S_R = Reproducibility standard deviation
RSD_R = Reproducibility relative standard deviation
R = Reproducibility value ($2.8 \times S_r$)
HORRAT = Horwitz ratio, an indication of the precision of the method.
* = With these samples, only 26 sets of results were supplied due to a misinterpretation of instructions

In this table the statistical evaluation of results from an interlaboratory evaluation of the methods is shown. Thirty-two laboratories (worldwide) were involved and sixteen samples (eight blind duplicates) were analysed.

STANDARD OPERATING PROCEDURE SUMMARY

Content of Amino Acids in Supplements and Related Products

1. Scope

This method is applicable to the quantification of individual amino acids in supplements and related products.

2. Principle

The amino acids are derivitized with 9-Fluorenylmethylchloroformate to form a chromatographable compound from a primary or secondary amine. The extract is analysed by HPLC-UV.

3. Definitions

3.1 FMOC - 9-Fluorenylmethylchloroformate

3.2 HPLC-UV – High Performance Liquid Chromatograph with Ultraviolet Detection.

3.3 MSDS – Material Safety Data Sheet

3.4 LCS – Laboratory Control Sample

4. Sample Requirements

Matrix	Sample Quantity	Storage Conditions	Hold Time
Supplement capsule/tablet	Minimum 20 tablets	Store at Room Temperature	1 year
Powder/ Raw material	Minimum 20 g	Store at Room Temperature	1 year

5. Internal Quality Control Requirements

Quality Control Check	Frequency	Acceptance Criteria (95% Confidence Interval)	Corrective Action
Calibration Standards	Every batch	$r^2 \geq 0.99$	Re-run Optimization
Method Blanks	Every batch	< MDL[1]	Re-run Re-analyse
Continuing Calibration Verification Standards (CCV)	Every 10 samples	80-120%	Re-calibrate
Duplicates	5%	As per QC Chart[2]	Re-calculate Re-analyse Re-run Optimization
Laboratory Control sample (LCS)	Every batch	70-130%	Re-run Optimization

1. MDL – Method Detection Limit.
2. Refer to GSOP71 for guidance on QC Data Evaluation Using Control Charting Techniques.

6. Method References

6.1 Einharsson, S., et.al. "*High-Resolution Reversed-Phase Liquid Chromatography System for the Analysis of Complex Solutions of Primary and Secondary Amino Acids*" American Chemical Society, 1986, Vol. 58, No. 8, pp 1638-1643.

6.2 US EPA 645

6.3 US EPA 8318

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